REMARKS

1. General Matters

1.1. The examiner is thanked for rejoining claims 90-92 with claims 73-89.

1.2. Claims 73-92 have been cancelled in favor of new claims 93-132.

1.3. A substitute abstract is enclosed.

1.4. The objections to claims 73-92 are mooted by the amendment.

2. Definiteness Issues (pp. 5-6)

The examiner questioned the following terms in claims 73-92:

(a) "expression signal" (73, 91, 92)

(b) "enzyme activity controlling assimilation of a nitrogen source" (73)

(c) "and/or (73)

(d) "as compared to the expression of the third enzyme activity when the third enzyme activity is associated with its native expression signal" (73)

(e) "operatively linked" (74)

(f) "gluta 30 mate" (80)

(g) "suitable" (90)

(a) The term "expression signal" is implicitly defined by the specification at P41, L5-6, as a sequence "directing expression" of the coding sequence. The expression signal is discussed in greater detail on pp. 52-53, which notes at P52, L13-17 that is preferably a regulatable promoter.

The examples disclose use, not only of promoters, but also of the PGK terminator (P60, L5). There are passing references to upstream activating sequences (UAS), enhancer elements and silencer elements at P52, L19-20.

There is basis for "regulatory nucleotide sequences" at P52, L26. This term is more commonly used in the art, and, like "expression signal", embraces promoters, terminators, enhancers, silencers, etc. A regulatory sequence may be upstream or downstream of the coding sequence, or within an intron.

New claims 93, 95, 112, and 132 recite "regulatory sequence" instead of "expression signal". New claim 130 recites that at least one of the contemplated regulatory sequences is a promoter.

(b) The exact phrase from claim 73 has not been retained. The term "controlling" is used in new claim 95. The term "assimilation" is no longer used.

The examiner questioned whether the term "enzyme activity" necessarily refers to a protein encoded by a nucleic acid. The increase enzyme activity of 93(i) is so limited by a later phrase of claim 93 which recites "wherein the increased enzyme activity is that of an enzyme encoded by a nucleic acid coding sequence...." Likewise 93(ii) recites that the reduced or eliminated enzyme activity of 93(ii) is one corresponding to a native activity which is that of a native enzyme encoded by a native nucleic acid coding sequence.

Please note that claim 93(ii) recites just "reduced", instead of "reduced or eliminated", because "eliminated" was redundant (effectively a 100% reduction). New claim 131 recites elimination.

It should also be noted that the increased enzyme activity of 93(i) can be one not native to the cell, cp. claim 101. Clause 93(i)(1) refers to increased enzyme activity resulting from an enzyme endogenous to said cell but produced under control of a promoter foreign to its coding sequence, while 93(i)(2) to increased enzyme activity corresponding to an enzyme exogenous to the cell. In the latter case, by definition there is no activity of the enzyme in question in the native cells, so any nonzero activity is an increase.

(c) The term "and/or" no longer connects the elements (i) and (ii) of old claim 73. The term is used, in a different context, in new claims 93 and 112. A phrase like "A and/or B" covers A alone, B alone, and both A and B. The term "and/or" appears in dictionaries and in many patent claims.

(d) New claim 93 plainly recites that the coding sequence is linked to a non-native promoter, and hence justifies the reference to a native promoter in the "as compared" limitation.

(e) The Examiner criticized operative linkage of different enzymatic operations. In the new claims, the term "operably linked" is used only in connection with nucleotide sequences.

(f) This was, as the examiner notes, a typo for "glutamate".

(g) The term "suitable" is not used in the new claims.

3. Description Issues (OA pp. 3-4)

Claims 73-92 were rejected for lack of written description. While the rejection applied to all of the claims, the Examiner did not specifically analyze any claim other than claim 73.

In essence, claim 73 recited use of a non-native promoter to increase "enzyme activity controlling assimilation of a nitrogen nutrient source". While there is reference to "first" and "second" enzyme activities, since these were linked by "and/or", and the claim was open in form, the claim effectively required that at least one enzyme activity be increased by expression signal replacement.

The claim also required that this be done in combination with reducing or eliminating a third enzyme activity, again by use of a non-native expression signal.

Claim 73 did not further identify either the enhanced enzyme activity(ies) or the reduced enzyme activity.

In contrast, new claim 93 specifies that the at least one increased enzyme activity be that of an NADH dependent enzyme catalyzing one of three specified reactions:

(a) 2-oxoglutarate + NH_3 + NADH → glutamate + NAD
(b) 2-oxoglutarate + glutamine + NADH → 2 glutamate + NAD and
(c) glutamate + NH_3 + ATP → glutamine + ADP + Pi

In addition, it specifies that the reduced or eliminated enzyme activity be one which (1) controls anabolic metabolism of ammonia as a nutrient source, and (2) is a natively present NAPDH-dependent glutamate dehydrogenase activity.

Basis for reaction (a) of 93(i) appears at P17, L4 in combination with P17, L16-18. Basis for reactions (b) and (c) appears at P17, L25 to P18, L2, and P71, L13-18.

In preferred embodiments, GLN1 and GLT1 are overexpressed, see P18, L30-31. See also P26, L32-P28, L3.

Basis for clause (ii) appears at P17, L15 and P28, L25-30; GDH1 is observed to be a NAPDH-dependent glutamate dehydrogenase at P18, L9-14 and 24-26.

It is noted that there are now functional linkages joining the claimed enzymatic activities. Reactions (a) and (b) have the common starting material 2-oxoglutarate, and both produce glutamate. Reaction (c) starts with glutamate and produces glutamine, which is a co-reactant in reaction (b). The glutamate of (a) and (b) is a substrate for the glutamate dehydrogenase of clause (ii).

The controlled nitrogen source, in the case of reactions (a)-(c) is clearly stated, and in the case of clause (ii) is, directly or indirectly, ammonia.

While claim 93 is not limited to a particular kind of microbial cell, the Examiner has not explained why the enzymatic activities in question could not be imparted to a broad range of microbial cells. However, if the rejection is maintained on this basis, the Examiner must explain why it should apply to claims 113 (microbial cell is a yeast cell), 114 (Saccharomyces,

Schizosaccharomyces or Pichia), 115 (S. cerevisiae TM19, DSM12276), and 116 (S. cerevisiae TN22, DSM12277).

Respectfully submitted,

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